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Single-Nutrient Effects on Immunologic Functions

Report of a Workshop Sponsored by the Department of Food and Nutrition and Its Nutrition Advisory Group of the American Medical Association

William R. Beisel, MD; Robert Edelman, MD; Kathleen Nauss, PhD; Robert M. Suskind, MD

• **Immune system dysfunction can result from single-nutrient deficiencies or excesses, alone or in combination with generalized protein-energy malnutrition. Acquired immune dysfunctions in man occur with deficiencies of iron, zinc, vitamins A and B_{12}, pyridoxine, and folic acid and with excesses of essential fatty acids and vitamin E. Additional micronutrients are important for maintaining immunologic competence in animals. Deficits or excesses of many trace elements and single nutrients thus have potential for causing immune dysfunctions in man. Since nutritionally induced immune dysfunction is generally reversible, it is important to recognize and identify clinical illnesses in which immunologic dysfunctions are of nutritional origin. Correction of malnutrition should lead to prompt reversal of acquired immune dysfunctions.**

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SEVERE multinutrient deficiencies lead to impaired immunocompetence. Acquired immune system dysfunction can also result from deficiencies, imbalances, or excesses of single nutrients.[1-4] Lymphoid tissue atrophy has long been known to accompany starvation or wasting illnesses. Only during the past decade has much clinical attention been given to the occurrence of impaired immunocompetence in patients who become malnourished.

Generalized malnutrition is most common in underdeveloped nations. It can also arise as a consequence of severe surgical or medical illness. Acquired immune system dysfunction is therefore seen in many hospitalized patients. Impaired immunocompetence increases susceptibility to respiratory, dermal, intestinal, or systemic infections and contributes to high mortality.

When it results from generalized malnutrition, acquired immune dysfunction is relatively easy to diagnose and reverse. Furthermore, simple clinical tests of cell-mediated immunity (such as skin testing with common recall antigens) may have prognostic value in severely malnourished patients.[2,3] The recognition of deficient immunocompetence can thus be important in many clinical fields.

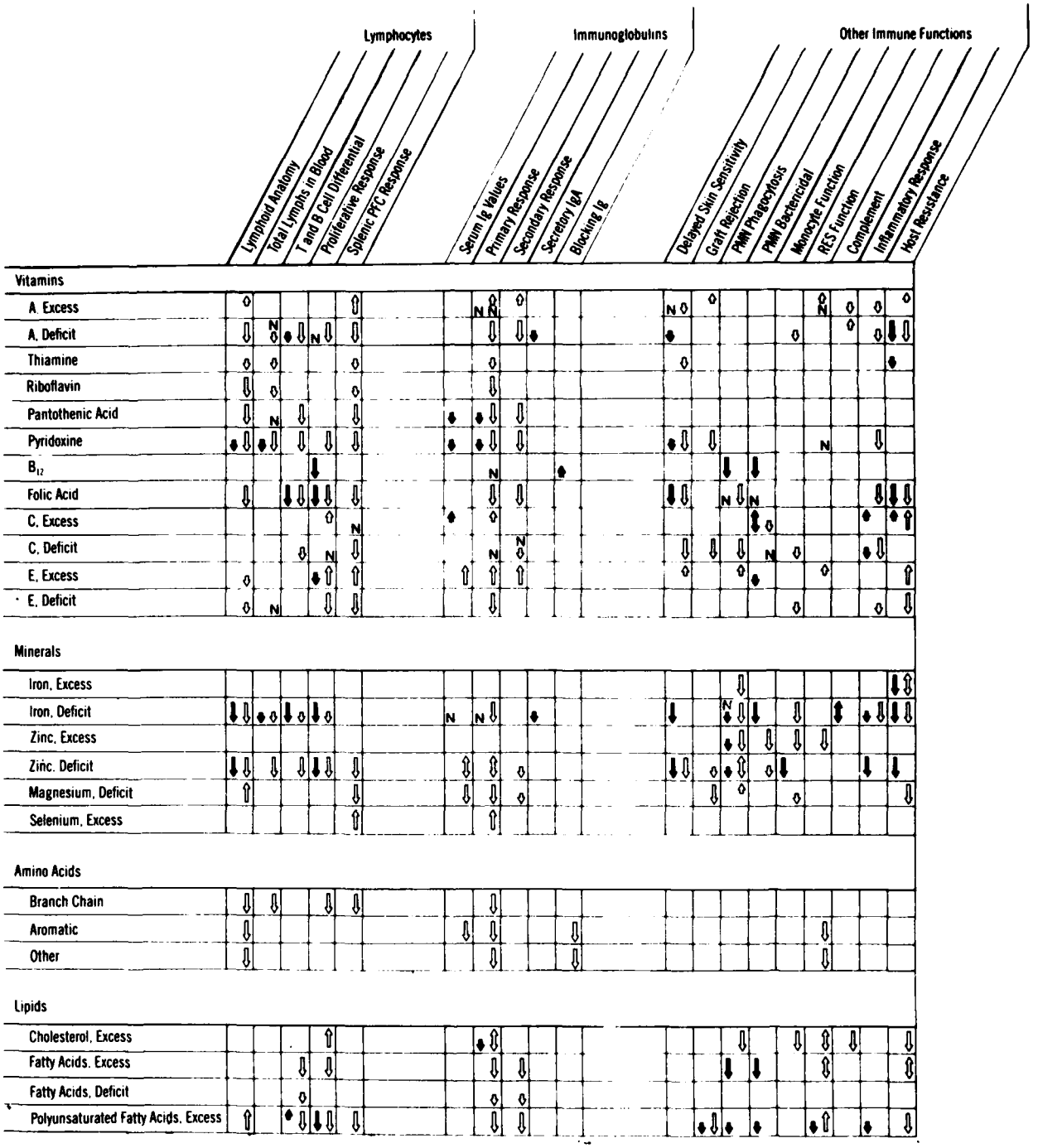
THE ROLE OF SINGLE NUTRIENTS

Most clinical studies of nutritionally impaired immunocompetence involve multiple deficiency states. Far less is known about the contribution of individual nutrients to immune system functions in man. In generalized malnutrition, it is virtually impossible to define causal relationships between individual nutrients and abnormalities in immune responsiveness. Severe deficiencies of protein and other energy sources influence the manner in which body cells use single essential nutrients. However, both animal data and clinical studies suggest that many individual

A complete list of publications assembled by workshop participants is available from the Department of Foods and Nutrition, American Medical Association, 535 N Dearborn St, Chicago, IL 60610 (Margarita Nagy).

From the US Army Medical Research Institute for Infectious Diseases, Fort Detrick, Frederick, Md (Dr Beisel); the National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Md (Dr Edelman); the Department of Nutrition and Food Science, Massachusetts Institute of Technology, Cambridge (Dr Nauss); and the Department of Pediatrics, University of South Alabama College of Medicine, Mobile (Dr Suskind).

Reprint requests to Department of Foods and Nutrition, American Medical Association, 535 N Dearborn St, Chicago, IL 60610 (Margarita Nagy).



Any reported change in immune function (top) associated with nutritional variable (left) indicated by direction and length of arrows. Solid arrows indicate human studies; open arrows, animal findings; N, normal findings; PMN, polymorphonuclear leukocyte; PFC, plaque-forming colonies; RES, reticuloendothelial system.

nutrients are important to immune competence.

This workshop was held to assemble information related to single-nutrient effects on the immune system, and to determine clinical applicability of the data. Major gaps exist in current knowledge (Figure); the literature is fragmentary and often limited to single reports. Data from laboratory animals are more complete than data from man, but no individual nutrient has been studied comprehensively for its effects on all measurable immune functions. Many older concepts and techniques are no longer considered valid for evaluating immune system complexities. The interrelationship of single nutrients and immunocompetence must be studied further, for both clinical application and basic knowledge.

Single-nutrient deficiencies, imbalances among individual nutrients, or marked excesses of single nutrients depress some immune functions (although a modest increase in the dietary intake of vitamins A and E and selenium may enhance certain immune functions in animals).

Immunologic changes in man occur with deficiencies of folic acid, iron, and zinc or with excesses of vitamin E or essential fatty acids.[1,2] Scattered clinical reports also document some immunologic changes with vitamin A, B_{12}, pyridoxine, or pantothenic acid deficiencies, and possibly with hypercholesterolemia.[2-4] Many individual vitamins, minerals, trace elements, amino acids, fatty acids, and cholesterol[1,2] influence immunologic mechanisms in animals.

WATER-SOLUBLE VITAMINS

The most important immunologic effects produced by B-group vitamins are seen with deficiencies of pyridoxine, pantothenic acid, and folic acid.[4] Deficiencies of vitamin B_{12} in man may be accompanied by defective immune functions. Deficiencies of thiamine, riboflavin, niacin, or biotin have little effect of immunocompetence.[4]

Pyridoxine Deficiency

A deficiency of pyridoxine depresses both cellular and humoral immunity in animals. Lymphoid tissues atrophy, delayed cutaneous hypersensitivity reactions fail to develop, and the expected rejection of skin transplants is inhibited. Impaired humoral responses are evidenced by poor antibody production after either primary or booster immunizations.[2,4] Populations of B lymphocytes and T lymphocytes fail to exhibit normal proliferative responses when stimulated in vitro by test mitogens or antigens. Thymic hormone activity is decreased. Volunteers with short-term experimental pyridoxine deficiency show reduced antibody responses to vaccines.[5]

Pyridoxine deficiency leads to impaired DNA and protein synthesis. The observed abnormalities in lymphocyte multiplication and immunoglobulin production appear to be secondary to these molecular defects.

Pantothenic Acid Deficiency

Pantothenic acid deficiency leads to depressed antibody responses after either primary or booster injections of vaccine antigens or heterologous RBCs. Deficiency of pantothenic acid thus appears to inhibit the stimulation of antibody-producing cells and their ability to produce new immunoglobulins.[4] Experimentally induced, brief pantothenic acid deficiency in man also reduces antibody responses to immunization.[5]

Other B-Group Deficiencies

Lymphocyte responses to mitogens are impaired and a modest reduction in the phagocytic and bactericidal capacity of neutrophils may be found in patients with primary pernicious anemia. Vitamin B_{12} deficiency cannot be produced in laboratory animals.

Folic acid deficiency depresses immune functions in both animals and man.[1,4] Patients with folic acid deficiency have an impaired ability to respond to skin-test antigens. Peripheral lymphocytes are not triggered by mitogenic stimulation in vitro, but neutrophil functions remain normal.

Guinea pigs and rats are extremely susceptible to folate deficiency. Animals lacking in folate demonstrate lymphoid atrophy, diminished WBC and neutrophil numbers, impaired cell-mediated immunity when tested in vitro, and reduced humoral response to injected antigens.

Isolated deficiencies of methionine and choline depress humoral immune functions in adult animals. Impaired resistance to bacterial infections, atrophy of lymphoid tissue, and defective T-cell-mediated immune function occur also when deprivation is initiated during the prenatal period.

Ascorbic Acid

Despite the popularity of large "prophylactic" doses of vitamin C, there are few data to suggest that ascorbic acid plays a role in lymphocyte function, although it does influence phagocytic cell migration and killing functions, as well as the healing of wounds.[4] The vitamin C content of WBCs may decrease during viral infections, pregnancy, and in elderly persons. Conventional doses of vitamin C may improve phagocytic function in children with congenital neutrophil defects such as the Chediak-Higashi syndrome.[1]

Vitamin C deficiency also reduces delayed cutaneous hypersensitivity responsiveness to skin-test antigens, but this is caused by an inability to develop a local inflammatory response rather than by an immunologic defect in the antigen recognition or processing functions of lymphocytes.

FAT-SOLUBLE VITAMINS

Vitamin A

Modest increases in dietary vitamin A enhance resistance to infection in animals and responsiveness to antigenic stimuli and accelerate the rejection of skin grafts. Vitamin A may also function as an adjuvant, if it is mixed with an antigen before its injection.

Vitamin A deficiency in animals leads to depletion of thymic lymphocytes, depressed lymphocyte responses to various mitogens, and an increased frequency and severity of bacterial, viral, and protozoan infections. The incidence of spontaneous infections is also said to increase in vitamin A-deficient humans.[1] Secretory IgA production may be impaired.[1,4] These effects may be related to the action of vitamin A in maintaining the composition of external cell membranes and surface glycoproteins and in favoring cell differentiation.

Vitamin E

The effect of vitamin E on the immune system has been studied in

both farm and laboratory animals.[8] Vitamin E deficiency depresses immunoglobulin responses to antigens, lymphocytic proliferative responses to mitogens and antigens, delayed dermal hypersensitivity reactions, and general host resistance. Attenuated live virus vaccines may become pathogenic in vitamin E-deficient puppies. On the other hand, in doses twofold to tenfold greater than minimal requirements, vitamin E has been found to enhance antibody responses to animal vaccines, to enhance delayed dermal hypersensitivity reactions, to accelerate the clearance of particulate matter by the reticuloendothelial system (RES), and to enhance host resistance and the ability to survive experimental infections.[8] In contrast, megadoses of vitamin E in healthy volunteers inhibit multiple immune functions.[9]

MINERALS

Divalent cations have important regulatory influences on external membrane functions of all body cells. Calcium and magnesium ions also participate in the activation of complement. Iron and zinc help to regulate immune functions in animals and man. Scattered reports indicate that other trace elements may also influence immune responsiveness. These include cadmium, chromium, copper, lead, manganese, and silica. Heavy-metal toxicity depresses immune functions.

Iron

Iron deficiency, often seen as an isolated nutritional problem, causes immune dysfunctions in large numbers of patients. Iron deficiency is accompanied by lymphoid tissue atrophy and impaired in vitro lymphocyte responsiveness to mitogenic stimulation.[10] Iron-deficient humans demonstrate impaired cutaneous hypersensitivity responses and defective macrophage and neutrophil functions. Not all investigators have found the same immunodeficiencies in iron-depleted patients, perhaps because of the coexistence of other deficiency states, concurrent or recent infections, the degree or duration of the iron deficiency in a given subject, or differences in the methods used for collecting samples and performing functional in vitro assays on cultured human cells.[11] Nevertheless, most studies suggest that the immune system of man is exquisitely sensitive to iron availability and responds adversely to deficiencies that are too small to lower hemoglobin values.[10]

Too much iron can also be deleterious and can saturate plasma iron-binding proteins. This increases the availability of iron for uptake by microorganisms and may lead to overwhelming sepsis. Severely malnourished patients with coexisting deficiencies of protein and iron exhibit very low concentrations of iron-binding proteins in plasma; they may experience a clinical activation of intracellular infections, such as malaria, tuberculosis, or brucellosis, during iron repletion therapy.[12]

Zinc

Zinc deficiency causes atrophy of lymphoid tissue and produces abnormalities in both cellular and humoral immunity.[13] Lymphocytes demonstrate a decreased in vitro response to mitogens and antigens and depressed T-killer cell activity. Humoral immune responses are inadequate. Delayed cutaneous hypersensitivity reactions and skin graft rejections do not occur in severely zinc-deficient animals, and thymic hormone activity is suppressed. Immature or precursor lymphocytes may be harmed more than the mature cells. Studies of severely zinc-depleted animals are complicated by profound anorexia and early death. However, the immune deficiencies can be reversed by restoring zinc.

Human neutrophilic functions are altered in vitro, if zinc is added to the incubation media. Excess in vitro zinc inhibits the bactericidal and phagocytic function of macrophages and neutrophils. Delayed cutaneous hypersensitivity responses are impaired in zinc-depleted patients. A local application of zinc sulphate to the skin restores responsiveness, apparently by direct absorption.[13] Zinc-deficient patients also show a restoration of immune functions when body zinc is replenished.

Selenium

A modest increase in dietary selenium, alone or in combination with vitamin E, appears to enhance immune responsiveness to vaccine antigens in animals.

Magnesium

Magnesium depletion is not known to produce immunologic impairment in man. However, unusual responses during prolonged magnesium deficiency in animals include hyperemia of the skin, eosinophilia, leukocytosis, degranulation of mast cells with release of histamine, histaminuria, thymic atrophy, and reduced humoral immune response to a variety of antigens.[14] Magnesium-deficient rats are not sensitized by brain-tissue antigens that induce allergic encephalitis in control subjects. Persistent leukocytosis in magnesium-deficient animals is often followed by malignant lymphoma-leukemia, myeloid leukemia, or malignant transformation of the thymus. At the same time, the occurrence of spontaneous or induced tumors is inhibited.

AMINO ACIDS

Dietary deficiencies of many single essential amino acids (phenylalanine, tyrosine, valine, threonine, methionine, cystine, or tryptophan) impair humoral antibody responses in mice but have little apparent effect on cell-mediated immunity. An excessive dietary intake of leucine, if sufficient to cause an amino acid imbalance, reduces the antibody response to immunization in animals. No reports in man document an association of single amino acid deficiency with loss of immune function.[2]

LIPIDS

Abnormalities in lipid intake or metabolism can initiate important changes in immunity. Experimental hypercholesterolemia in animals tends to decrease resistance to bacterial or viral infections or to tumors. Suppression of inflammatory infiltrates and impairmant in phagocytic cell and RES functions and in primary antibody responses have been reported.[2] Perturbations in immune function are presumed to be mediated by increases in cell membrane cholesterol content and altered membrane fluidity.

A deficiency of essential fatty acids depresses both the primary and secondary antibody responses to both T-cell dependent and independent antigens in mice. Such an effect has not been reported in man. On the other hand, excess polyunsaturated fatty acids (PUFAs) produce widespread immunologic defects in laboratory animals, including lymphoid tissue atrophy, diminished delayed cutaneous hypersensitivity, and depressed T-cell immune responsiveness to antigenic stimulation. When infused, excess PUFAs cause lymphoid necrosis, impaired RES function, and a depressed rejection response to heterologous grafted tissue. If PUFAs are added in vitro to cell culture media, the phagocytic capacity of neutrophils is impaired and the proliferative responsiveness of lymphocytes is inhibited. However, this inhibition may result from direct toxic effects of PUFA on cultured cells. In patients given conventional immunosuppressive therapy, a high-PUFA diet caused an additional delay in the rejection time of renal transplants.[15]

Saturated fatty acids can serve as adjuvants for a variety of antigens and, in addition, appear to activate macrophages.

DIAGNOSTIC CRITERIA

General Concepts

Immune dysfunction may be present in every wasted or debilitated patient. It is generally more difficult to diagnose single-nutrient malnutrition than generalized protein-energy malnutrition. Although the coexistence of both immunologic and nutritional abnormalities does not mean that a cause-and-effect relationship exists, such would be suggested if an immunologic defect disappeared rapidly following clinical elimination of the nutritional problem. Thus, a therapeutic attempt to correct a nutritional abnormality can also serve as a diagnostic maneuver.

While single-nutrient deficiencies may contribute to generalized malnutrition, they can also lead to immunoincompetence in patients whose general nutritional status seems relatively normal. Single-nutrient deficiencies occur most commonly in patients with chronic anemias, alcoholism, recurrent or chronic diarrhea, chronic renal, liver, or biliary disease, regional enteritis or other malabsorptive states, or after intestinal bypass surgery. Patients receiving long-term hemodialysis or intravenous alimentation may incur unsuspected deficits or excesses of single nutrients. Other "at-risk" groups include the elderly, pregnant women, low-birth-weight infants (both small-for-age and preterm), and children with growth retardation, congenital aminoacidurias, or recurrent bacterial or yeast infections. Surprisingly, obese persons may manifest micronutrient deficiencies, particularly zinc and iron. Drugs and other therapeutic measures may induce single-nutrient abnormalities, such as those caused by folic acid antagonists, phenytoin sodium (folate deficiency), and isoniazid therapy (pyridoxine deficiency).

A single-nutrient deficiency or excess may develop in patients who are food faddists or in those who receive unusual therapeutic diets. Many persons currently consume large quantities of certain vitamins (A, B_{12}, C, E, niacin, or pyridoxine), minerals (zinc or selenium), or other single nutrients, including tryptophan, lecithin, or PUFA. It is therefore important to determine if a patient is following an unusual diet (prescribed or self-imposed) or is ingesting large quantities of a single nutrient.

Single-Nutrient Testing

Confirmation of a single-nutrient deficiency or excess may require reference laboratory facilities. A complete battery of clinical tests for nutrient-related anemias is possible in most major facilities. Iron status may be evaluated by determining hemoglobin level, RBC count, hematocrit reading, serum iron level, total transferrin or iron-binding capacity, and by the presence of iron in marrow cells. Radioimmunoassays of serum ferritin serve to assess tissue iron stores. Vitamin B_{12} can be measured in serum and its absorption evaluated by the Schilling test. Folate measurements can be performed in serum or RBCs.

Easily measured trace elements include zinc and copper; nickel and chromium determinations are more difficult. Most other trace elements can be measured with accuracy only in specialized laboratories. Assays for most vitamins, individual free amino acids, and individual fatty acids usually require specialized laboratory facilities.

Immunologic Evaluation

Some immunologic studies can be performed easily. Normal adults demonstrate delayed cutaneous hypersensitivity to skin tests with many ubiquitous antigens, such as monilia, phytohemagglutinin, streptokinase-streptodornase, or trichophyton. In some populations, mumps and tuberculin sensitivities are also common. Anergy exists if none of the common recall antigens elicits a delayed cutaneous hypersensitivity reaction. An anergic patient could be studied further by purposeful dermal sensitization with an unfamiliar new antigen, dinitrochlorobenzene, to test cell-mediated immunity. An initial application evaluates the ability to generate a localized inflammatory response, to become sensitized (ie, to develop immunologic memory), and a second application quantitatively tests recall.[1] While these studies can have diagnostic value, appropriate nutrient therapy should not be withheld simply to achieve research goals.

Measurements of total IgG, IgM, IgA, IgE, or complement concentrations in serum tell little about nutritionally induced immunoincompetence. On the other hand, a depressed IgA content in body surface fluids and secretions (eg, saliva or milk) can be an important finding. The enumeration of subsets of human lymphocytes may also assist in evaluating immune status. This is done by determining if there are immunoglobulins on their surface membranes, if sheep RBCs adhere to form rosettes, or if they can kill foreign cells.

Lymphocyte proliferative responses to mitogens or test antigens can now be measured in many laboratories. Such assays require that lymphocytes from the peripheral blood be gathered, cultured in vitro, and tested over a period of several days to determine whether appropriate mitogens or antigens will stimulate them. Proliferative responses are quantified by determining the uptake of radiolabeled thymidine, a nucleic

acid precursor. In vitro assays can be performed in specialized laboratories to determine the chemotactic, phagocytic, and bactericidal capacities of neutrophils, as well as the ability to activate their hexosemonophosphate shunts.

Other participants in the workshop were Abraham E. Axelrod, PhD; Surendra Baliga, PhD; Ranjit K. Chandra, MD; Gabriel Fernandes, PhD; Allan L. Forbes, MD; Pamela J. Fraker, PhD; Philip Frost, MD, PhD; Raymond M. Galt, MD; George M. Hass, MD; Grant H. Laing, MD; Roger M. Loria, PhD; Margarita Nagy, MS, RD; Cheryl F. Nockles, PhD; Ronald D. Schultz, PhD; Gloria J. Troendle, MD; and Joseph J. Vitale, ScD, MD.

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